The Effect of Ethylenediaminetetraacetic Acid (EDTA) and Pepsin Enzyme Addition on The Characteristics of Yellowstripe Fish (*Selaroides Leptolepis)* Collagen and Gelatin

Juan Suhut Pradana[1], Nadya Aulia Warsito[1], and Ayu Dahliyanti[1*]

{juansuhutpradana@gmail.com[1], nadyaauliawarsito@gmail.com[1], ayu.dahliyanti@universitaspertamina.ac.id}

Department of Chemical Engineering, Universitas Pertamina. Jl. Teuku Nyak Arief, Simprug, East Jakarta 12220

Abstract. The importance of collagen and gelatin has been increasing recently due to their wide applicability in various fields. Marine organisms provide promising alternatives as both collagen and gelatin sources compared to their traditional bovine and porcine counterparts due to better properties, high abundance, and health and religious reasons. In this research, collagen and gelatin were acid-extracted from the skin of Yellowstripe fish (*Selaroides leptolepis*) with the addition of Ethylenediaminetetraacetic Acid (EDTA) and pepsin enzyme. Fourier-transform infrared spectroscopy (FTIR) showed that all as-extracted products contain all characteristic functional groups of collagen or gelatin. The addition of 5 wt.% of Pepsin increased the yield of collagen and gelatin to the optimum values of 7.19 wt.% and 3.57 wt.%, respectively. Ethylene Diamine Tetra-acetic Acid (EDTA) pretreatment successfully lowers the mineral content down to 0.27 wt.% for collagen and 1.39 wt.% for gelatin. The Yellowstripe fish collagen and gelatin were stable until the denaturation temperature of 59.90℃ and 74.66℃.

Keywords: Collagen, gelatin, Yellowstripe fish, Ethylene diamine Tetra-acetic Acid, EDTA, Pepsin.

1 Introduction

Collagen accounts for up to 30% of the total protein in the human body. However, collagen content decreases by 0.5%-1% with age, so that at 30-40 years, humans only have 60% of the total natural collagen [1] . As a result, supplementary collagen consumption is in high demand due to its numerous benefits, such as in pharmaceutical and cosmetic industries because of its excellent biocompatibility, easy biodegradability, and weak antigenicity [2]. Collagen reacts with water to produce gelatin. Gelatin is also widely used by the biomedical and food industries to make jelly, ice cream, tablet coating, capsule shells, emulsifiers, and many others [3] [4]. Until now, collagen and gelatin are sourced primarily from land-based mammalian (cows, pigs, etc.) [5]. However, bovine or porcine collagen and gelatin have been less preferred due to the potential transmission of dangerous diseases such as brucellosis, anthrax, bovine spongiform encephalopathy (BSE), foot and mouth disease (FMD), and avian influenza as well as religious and other dietary restrictions [6].

ICO-SEID 2022, November 23-24, Jakarta, Indonesia

DOI 10.4108/eai.23-11-2022.2338888

Therefore, marine animals such as fish, sponges, jellyfish, octopus, etc. are now attracting a lot of attention as the alternative source of collagen and gelatin [7].

Collagen and gelatin can be extracted from marine animals using chemical or enzymatic method [8]. The collagen extraction process includes a section of preparation, pretreatment, extraction, and salting out. There are two common methods used in collagen extraction which are chemical and enzymatic [1]. In the chemical method, acid (acetic acid, citric acid, or hydrochloric acid) or alkali (sodium hydroxide, lime, sodium carbonates) are used to extract protein-containing collagen. Meanwhile, in the enzymatic method, protease enzymes such as pepsin, trypsin, and papain are commonly used. Studies show that acetic acid is the most promising solvent since it can cleave the hydrogen bond in the triple helix structure of collagen [9] [10]. Collagen has a strong bond in the polypeptide area; thus, it cannot be hydrolyzed by acids alone. So, we require the addition of enzymes to cleave the molecular bond in telopeptide crosslinks and influence the solubilization of collagen [11]. As reported by Jamilah [12], the addition of pepsin enzyme was able to increase the yield of collagen extracted from white snaapper (*Lates calcarifer*) up to 44%. Collagen extraction was also carried out from *Nibea japonica* fish using acid and pepsin with a yield of up to 84.85% by mass [13]. Pepsin-soluble collagen from blacktip shark cartilage has a yield of 10.30% and an ash content of 0.70% [14]. Similarly, collagen synthesized from dry haruan scales is 1.94% with an ash content of 0.66% [15]. Then, enzymatic extraction with pepsin enzyme was also carried out to obtain gelatin from cuttlefish skin, producing yields up to 9.22% by mass [16] The optimum condition of pepsin is generally at a temperature approximation of 20°C and a pH of 2-3 [17].

Yellowstripe fish (*Selaroides leptolepis*) has the potential to be an alternative source of collagen and gelatin due to its high protein content of 22,06% per 2 grams of fish, low price, and abundance [18]. This fish is classified as a mezzo-pelagic fish, which lives near the surface and on the seafloor [19]. Yellowstripe fish habitat is found in Indonesian waters such as Banda Sea, Padang, Sibolga Waters, Weh Island, Makassar, and Bulukumba. According to the Statistics Agency of the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (KKP), the total fish production in 2018 was 226,675.79 tons with a price of Rp. 30,000 per 1 kg [20].

In our previous research, collagen produced from the skin of Yellowstripe fish by a combination of acetic acid and pepsin extraction had a high yield of 18.64 mass % [21]. However, the ash content was still as high as 15.87%, which does not yet fulfill the Indonesian National Standard (SNI, SNI 8076:2014, SNI 01-3735-1995) for commercial collagen. Ash content represents the concentration of minerals and metallic compounds (as well as heavy metals) in the products which may originate from the pollution in the marine environment and being absorbed by marine organisms. Lower ash content will ensure the safety of collagen and gelatin products. Therefore, in this research, we introduce the addition of EDTA as an advanced demineralizing and metal-chelating agent to the extraction process and will investigate its effect on the moisture and ash content of collagen and gelatin products. We hope this approach can be used as a basis to support an economically feasible production of commercial collagen and gelatin with high purity from Yellowstripe fish.

2 Materials and method

2.1 Materials

Yellowstripe fish was purchased from a traditional market in South Jakarta. Sodium hydroxide (NaOH) 0.1 M solutions (Merck), ethylene diamine tetra-acetic (EDTA) 0.5 M solutions, glacial acetic acid (CH_3COOH) 0.5 M (Merck), NaCl powder (Pudak Scientific), pepsin enzyme solid powders are used without further purifications.

2.2 Methods

Collagen Extraction. Yellowstripe fish skins were separated from the body and washed with distilled water. Samples are also cut into small sizes using blenders with distilled water (15 w/v). Solid and liquids were separated using a refrigerated centrifuge at 10.000 rpm for 20 mins at 4℃. To dissolve non-collagen contents, such as mineral salts and other proteins, a 0.1 M sodium hydroxide (NaOH) solution was used with fish skin to solvent (solid to liquid) ratio of 1:10 (w/v). The dissolving time was 6 h, with a fresh solvent change at the 3rd hour, while being stirred continuously at a temperature of 4°C. The procedure was repeated once. After that, the skin was demineralized using a 0.5 M Ethylenediaminetetraacetic Acid (EDTA) solution with a fish skin-to-solvent ratio of 1:10 (w/v) for 24 hours, with a fresh solvent change at the 12th hour. The remaining solids were washed with distilled water until the pH reached 7-8 (neutral). Pretreated skin was then immersed in 0,5 M acetic acid (CH_3COOH) at a 1:10 (w/v) ratio, with the addition of 0%, 1%, and 5% pepsin by mass for 24 h. Then, the mixtures were centrifuged at 12,000 rpm for 10 mins at 4˚C, and the supernatants were collected. Supernatants are the soluble collagen fraction. Collagen was isolated from the supernatant by adding 2 M NaCl solution. The suspension of collagen was then filtered using filter paper.

Gelatin Extraction. The distilled water was added to the previously filtered collagen with a ratio of 1:2 (w/v) and then constantly heated at 70℃ for 1.5 hours to promote the transition to gelatin. After that, the solids were filtered using filter paper and dried inside an atmospheric oven at 50℃ for 18 hours.

Characterization. The collagen and gelatin were characterized by Fourier Transform Infrared Spectroscopy (FTIR, Thermo Fisher Scientific iS 5 using the KBr pellet method to identify the functional groups inside the samples. The wavenumber used ranged from 65 to 4000 cm^{-1}. A UV-Vis spectrophotometer (Thermo Fisher Scientific Genesys) is set at the wavelength of 200 to 400 nm to obtain the spectra of collagen and gelatin. A Differential Scanning Calorimetry (DSC, 250 TA Instruments) was used to analyze the denaturation temperature of collagen and gelatin with temperature scan intervals of 30 to 200℃, a nitrogen atmosphere, and a 10℃ per 2 mins heating rate. The yield of the collagen and gelatin in wt% is calculated using the following formula:

$$\text{Yield (\%)} = \left[\frac{\text{Dry collagen/gelatin mass (g)}}{\text{Yellowstripe fish skin mass (g)}}\right] \text{x } 100 \qquad (1)$$

For moisture content analysis, 0.2g of collagen or gelatin samples were heated inside an air oven at 105ºC for 3h. The mass of samples before and after heating was compared. Moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \left[\frac{\text{Initial} - \text{Final sample mass (g)}}{\text{Initial sample mass (g)}}\right] \text{x } 100 \quad (2)$$

For ash content analysis, 0.2g of collagen or gelatin samples were burned inside a furnace at 600ºC for 4h. The mass of samples before and after burning was compared. Ash content was calculated using the following formula:

$$\text{Ash content (\%)} = \left[\frac{\text{Initial} - \text{Final sample mass (g)}}{\text{Initial sample mass (g)}}\right] \text{x } 100 \quad (3)$$

3 Results and discussion

Collagen and Gelatin Yield. The visual images of the as-extracted collagen and gelatin from Yellowstripe fish skin are displayed in **Figure 1** and **Figure 2**. Collagen has a white to yellowish a color with a powdery texture. Whereas the gelatin is more brownish with a rubbery texture. No significant color differences were observed on the products obtained from different extraction parameters.

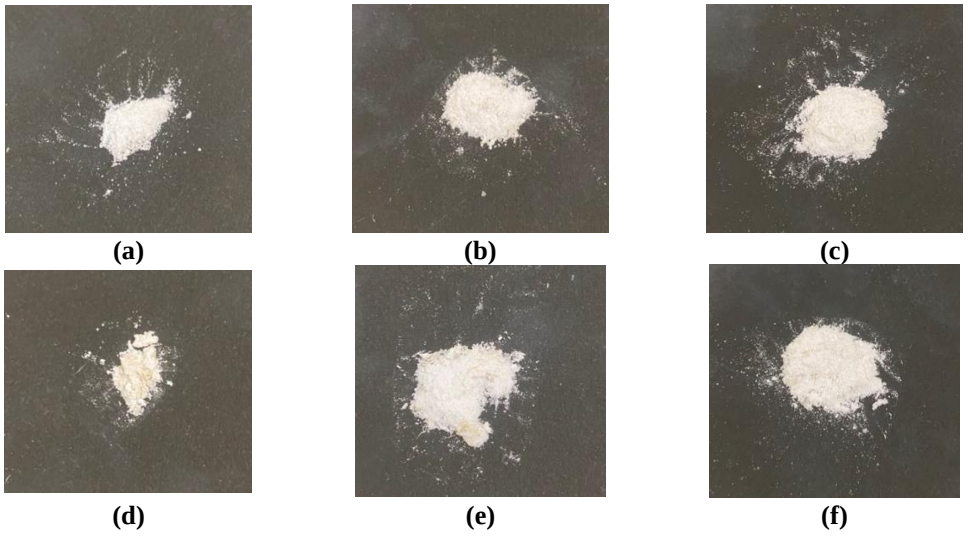


Fig. 1. The collagen extracted with the addition of (a) EDTA and 0% pepsin, (b) EDTA and 1% pepsin, (c) EDTA and 5% pepsin, (d) No EDTA and 0% pepsin, (e) No EDTA and 1% Pepsin, (f) No EDTA and 5% pepsin.

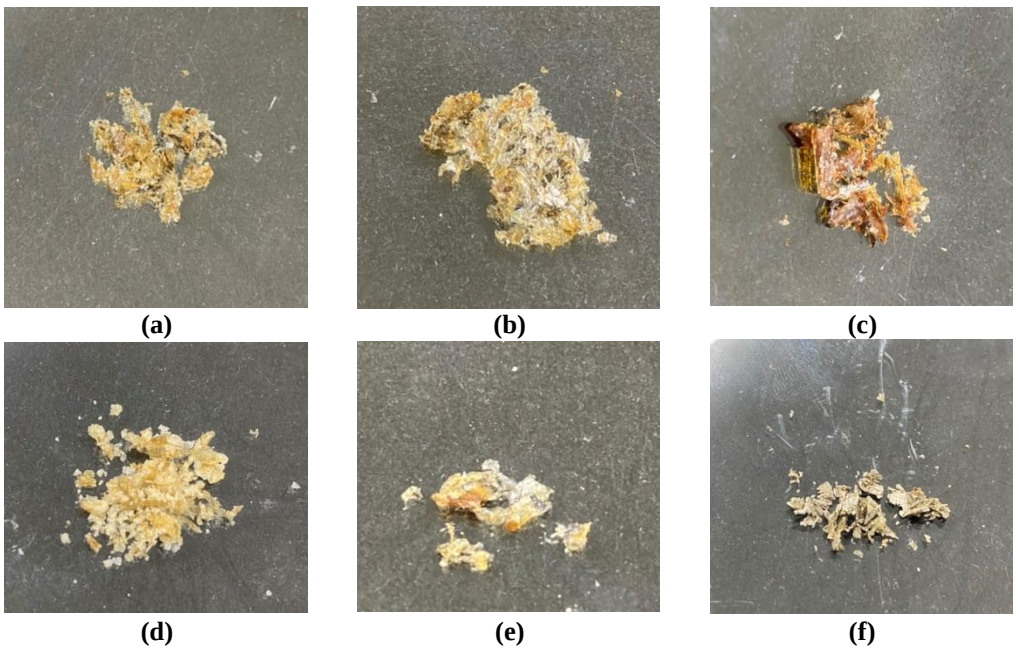
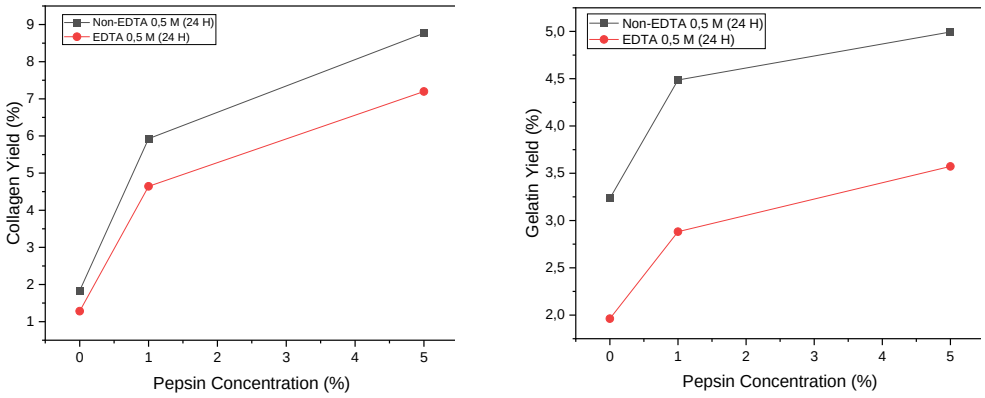


Fig. 2. The gelatin extracted with the addition of (a) EDTA and 0% pepsin, (b) EDTA and 1% pepsin, (c) EDTA and 5% pepsin, (d) No EDTA and 0% pepsin, (e) No EDTA and 1% Pepsin, (f) No EDTA and 5% pepsin.

As seen in **Figure 3**, collagen and gelatin yields increase with the addition of a higher concentration of pepsin enzyme during extraction. The pepsin enzyme helps break down the strong intermolecular covalent bonds in the telopeptide region, increasing the solubility of collagen [22]. In addition, pepsin can hydrolyze non-collagen proteins efficiently without damaging the triple helix structure, thus increasing the yield and purity of collagen and gelatin [23].



(a) **(b)**

Fig. 3. Yellowstripe fish skin (a) collagen (PSC) and (b) gelatin from Yellowstripe fish skin yield at various pepsin concentrations, with and without EDTA.

Pepsin is a class of proteases which has capability to attack protein complex bonds and degrading fish skin tissue, causing skin fibers to decrease, shorten and even break. When carrying out the extraction process, an optimum concentration of pepsin enzyme is necessary to produce a maximum yield [24]. Higher concentration of pepsin enzyme will increase the pepsin soluble collagen (PSC) yield due to wider reaction area and higher number of contact sites amongst Yellowstripe fish and pepsin [25]. However, when the pepsin concentration exceeds the maximum limit, the telopeptides will undergo a breakdown, which will reduce the extraction yield and begin to degrade the dissolved collagen molecules [26]. In addition, excessive pepsin can also create a hydrophobic-hydrophobic interaction between the enzyme on the Yellowstripe fish skin over the enzyme in the solvent. This will impact the ionic strength therefore reducing the PSC yield and moisture content [27].

The yield of collagen also depends on the fish species, the mass of the fish skin, and the protein content [28]. When using the skin of giant salamander fish, the collagen yield was found to be 0.1% mass [29], while it was 1% mass for silver carp skin [14]. At the current experimental condition, the optimum concentration of pepsin for collagen and gelatin extraction from Yellowstripe fish skin is 5%, with the maximum yield of 8.76% and 4.99% for collagen and gelatin, respectively. Although still following a similar trend, advanced demineralization using EDTA solution produced collagen (7.2%) and gelatin (3.57%) with a lower yield. This condition is mainly affected by the further removal of mineral contents such as arsenic, magnesium, calcium, mercury, and cadmium by EDTA [30].

Moisture and Ash Content Analysis. Moisture content is one important aspect of food quality standard because it is related to the safety and shelf life of the commodities [31]. The presence of moisture will support microbial or parasitic activities which result in the shorter shelf life of collagen

and gelatin products [21]. According to Fig.4, the moisture content decreases along with the increase of pepsin concentration, ranging from 9.87% to 6.90% for collagen, and from 12.14% to 5.08% for gelatin. Overall, the moisture content of the obtained samples has met the requirements of the Indonesian National Standard (SNI, SNI 8076:2014, SNI 01-3735-1995), which stated that the value must be lower than 12%. Concentration of pepsin has stronger influence on moisture content due to hydrolysis reaction of protein. Enzymes need water in order to break the telopeptide area bonds and intermolecular cross-links [28]. The water requirement in the process will be higher thus reducing the water content of the products [32].

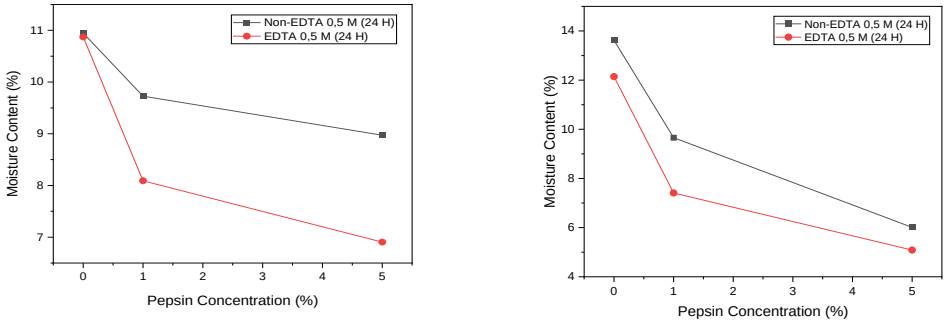


Fig. 4. Moisture content of (a) collagen and (b) gelatin from Yellowstripe fish skin at various pepsin concentrations, with and without EDTA.

The minerals will diffuse to the acid solvent phase when the extraction stage occurs, then trapped inside the gel-like network in salting out stage, resulting in collagen and gelatin having significant ash content [34]. The types of mineral metal that have the potential to become ash are As, Ca, Cd, Fe, Hg, Mg, and Tb compounds, while majority of the salts are in the form of NaCl [35].

As displayed in **Figure 5**, the ash content of samples which was pretreated with EDTA were significantly lower than the ones without EDTA pretreatment. Lone electron pairs in EDTA molecules will capture metal cations to form complex metallic compounds through coordinate covalent bonds. The addition of pepsin does not affect the ash content. Some specific minerals or metal ions such as Fe^{3+} and Zn^{2+} might form a complex with substrate and active side enzyme as a cofactor for catalytic reaction. However, pepsin cannot take chelating action to remove the ions, so it has no direct impact to ash content [33]. The highest ash contents are 0.2682% at 5% pepsin for collagen and 1.44% at 1% pepsin for gelatin. These results indicate that collagen and gelatin extracted from the skin of Yellowstripe fish have met the requirements of the Indonesian National Standard (SNI, SNI 8076:2014, SNI 01-3735-1995) which stated that the ash content must be <1% for collagen and <3.25% for gelatin. As a comparison, the ash content of the collagen extracted from herring scales using EDTA-2 Na 0.5 M was 0.66% [15]. This shows that the ash contents are also influenced by the type of marine organisms as well as the condition in their habitat.

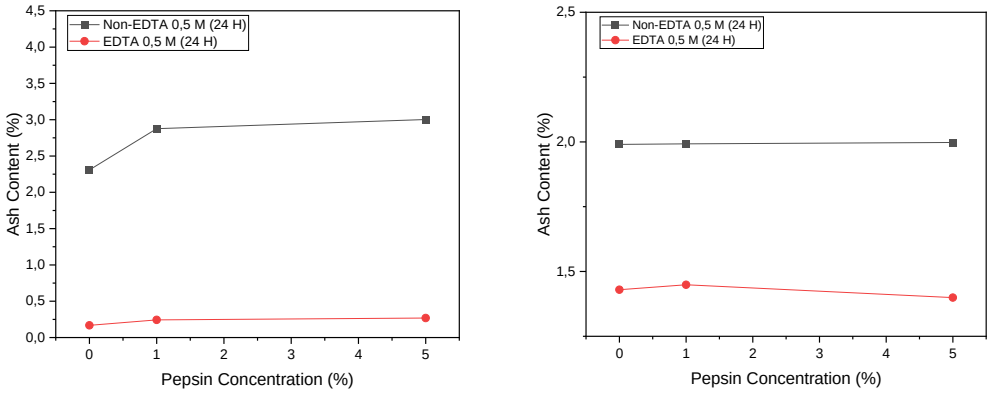
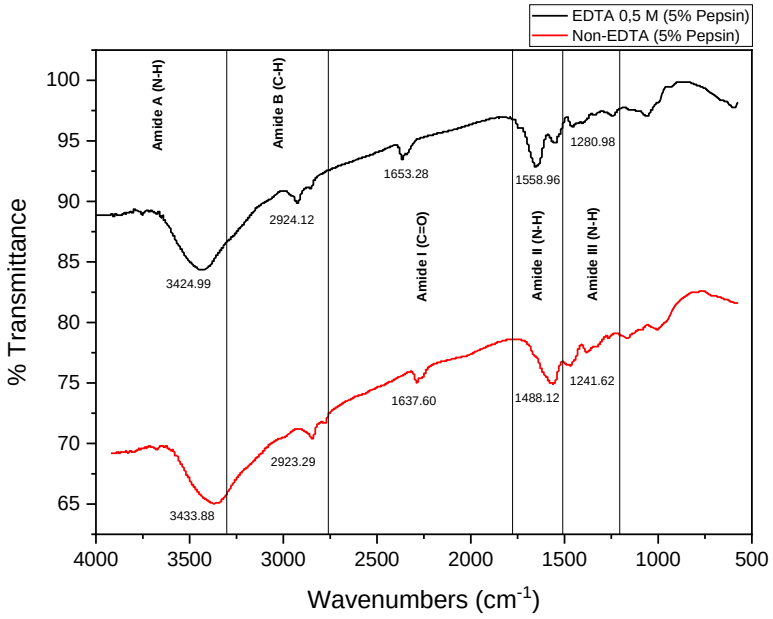


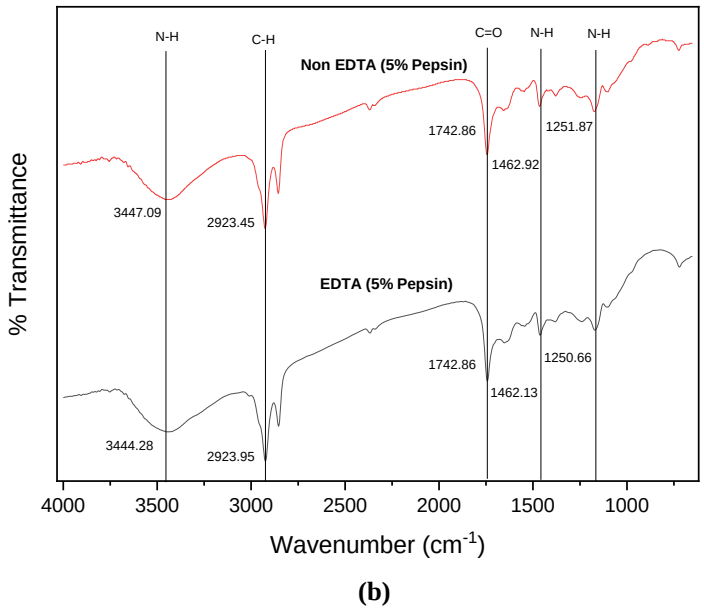
Fig. 5. Ash content of (a) collagen and (b) gelatin from Yellowstripe fish skin at various pepsin concentration, with and without EDTA.

Fourier Transform Infrared (FTIR) Spectra. There are about 29 different type of collagen that have been identified as type I-XXIX [36]. Collagen type I, which is found in the human body, can also be isolated from marine organisms [37]. Type I collagen can be identified by its characteristic functional groups of amide A, amide B, amide I, amide II, and amide III bonds [23].

As shown in **Figure 6 (a)**, those characteristic peaks can be found in FTIR spectra of samples extracted with 5% pepsin, with or without EDTA, confirming the Yellowstripe fish collagen as type I. N-H stretching vibration which represents amide A group was detected in wavenumber 3,400-3,440 cm-1 [34]. CH_2- asymmetrical stretch of amide B was detected at 2,915-2,935 cm^{-1} [17]. The stretching vibration activity of the C=O bond in the polypeptide region gives rise to the absorption of the amide I group at 1,600-1,660 cm^{-1} [38]. Amide II groups consist of N-H bonds and C-N asymmetric stretching vibration which can be identified at 1,480-1,575 cm^{-1} [39]. The last characteristic group, amide III, is a complex interaction between C-N and N-H bonds to produce absorption of CH_2 bonds. Amide III group was detected at wavenumber 1,200-1,400 cm^{-1} [26]. Furthermore, the ratio of the amide III wavenumber to 1,454 cm^{-1} were 1.13 (with EDTA) and 1.17 (without EDTA), suggesting that the collagen triple helix structure is intact [39]. EDTA can chelate mineral ions without cleaving and breaking the triple helix structure of Yellowstripe skin collagen [40]. Therefore the FTIR spectra of products have no significant difference with each other [41]. While in **Figure 6 (b)**, since gelatin is the derivative of collagen, the same characteristic functional groups can also be detected at similar ranges of wavenumber. Amide A peak tends to join the CH_2 peak when the carboxylic acid groups are in stable intermolecular bonds at lower temperature [42][43]. So that the steeper peak of gelatin compared to collagen is possibly due to the sequential high temperature process of gelatin extraction [44].


EDTA 0,5 M (5% Pepsin)
Non-EDTA (5% Pepsin)
Amide A (N-H)
Amide B (C-H)
Amide I (C=O)
Amide II (N-H)
Amide III (N-H)
3424.99
2924.12
1653.28
1558.96
1280.98
3433.88
2923.29
1637.60
1488.12
1241.62
% Transmittance
Wavenumbers (cm-1)
100
95
90
85
80
75
70
65
4000
3500
3000
2500
2000
1500
1000
500

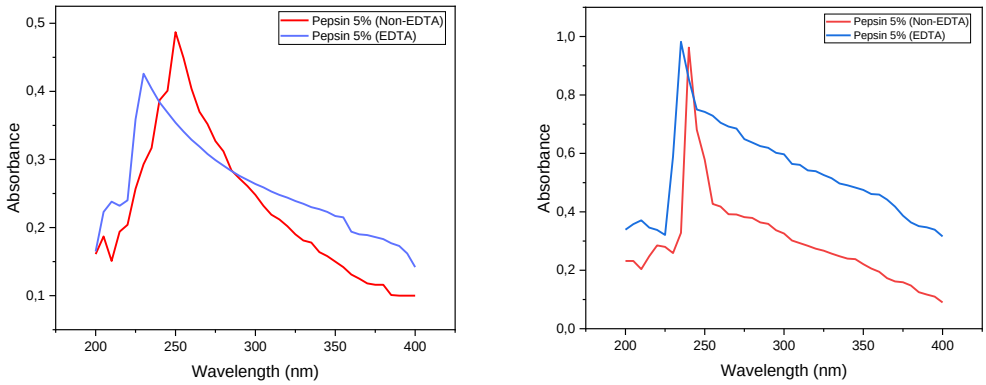
(a)



(b)

Fig. 6. FTIR spectra of the (a) Collagen and (b) Gelatin from Yellowstripe fish skin at 5% pepsin, with and without EDTA.

UV-Vis Spectrophotometry. Previous researchers reported that the maximum absorbance peak of collagen or gelatin is around 210-240 nm [32] [45]. From UV-Vis spectra in **Figure 7 (a)** and **Figure 7 (b)**, Yellowstripe fish collagen and gelatin which were extracted with EDTA and 5% enzyme have fit into this category, with maximum wavenumber of 230 nm respectively [46]. This is quite similar with collagen from catfish and ornate threadfin, with maximum wavenumber of 232 nm and 230 nm, respectively [47]. On the other hand, Yellowstripe fish collagen and gelatin which were extracted without EDTA have higher maximum wavenumber, indicating the presence of some impurities. This proves that EDTA plays an important role in producing collagen and gelatin with a high purity, respectively. This is quite similar with collagen from catfish and ornate threadfin, with maximum wavenumber of 232 nm and 230 nm, respectively [47]. On the other hand, Yellowstripe fish collagen and gelatin which were extracted without EDTA have higher maximum wavenumber, indicating the presence of some impurities. This proves that EDTA plays an important role in producing collagen and gelatin with a high purity



(a) **(b)**

Fig. 7. UV Spectra of (a) collagen and (b) gelatin from Yellowstripe fish skin at 5% pepsin, with and without EDTA.

Differential Scanning Calorimetry (DSC) Analysis. Collagen and gelatin are sensitive to temperature changes because they can experience structural damage or denaturation, resulting in the loss of nutritional benefit. In collagen, high temperature can damage the triple helix structure, which is the main characteristic of collagen, to single helix [1]. On the other hand, gelatin will undergo denaturation process to form semiglutin and hemkolin [48]. Measuring the denaturation temperature is important to determine the correct storage condition and shelf life of the products.

As displayed in **Figure 8 (a)** and **Figure 8 (b)**, collagen and gelatin which was extracted with 5% pepsin and 0.5 M EDTA addition have the denaturation temperatures of 59.90 °C and 74.66℃, respectively. This is quite high considering the denaturation temperature of marine collagen is 35-55ºC [49] .We believe that this is due to the fact that Yellowstripe fish lives in shallow, warm waters. As comparisons, pepsin soluble collagen from cold water fishes such as sharpnose stingray (31.76ºC), black carp (36ºC), and channel catfish (36 ºC) have lower denaturation temperatures [17] [50] [51]. While collagen from seabass, which lives in warm water of Mexico, also has a high denaturation temperature of 79 ºC [26]. Similarly for gelatin, the denaturation temperature from Yellowstripe fish is also higher compared to stingray (41.4 ℃) and salmon (45℃) [52] [53].

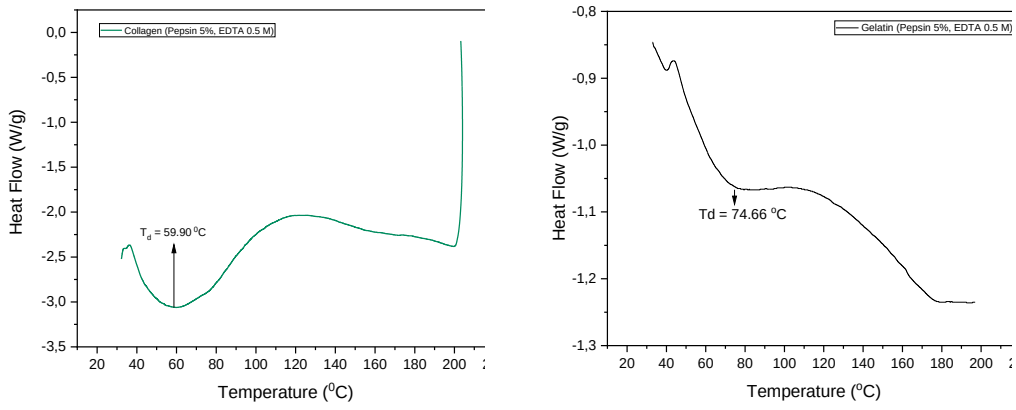


Fig. 8. DSC Thermogram of (a) collagen and (b) gelatin from Yellowstripe fish skin with 5% pepsin and EDTA.

4 Conclusion

The extraction of collagen and gelatin from the skin of Yellowstripe fish with the addition of the pepsin enzyme is proven to increase yield and reduce moisture content. Whereas the addition of EDTA will reduce ash content, thus improving the purity of collagen and gelatin. At this moment, refinement in extraction conditions as well as further testing such as proximate and physicochemical properties analysis are still needed. However, this experiment has already shown promising results which can support the commercial production of collagen and gelatin from Yellowstripe fish in the future.

References

[1] L. Salvatore *et al.*, "Marine collagen and its derivatives: Versatile and sustainable bio-resources for healthcare," *Materials Science and Engineering: C*, vol. 113, p. 110963, Aug. 2020, doi: 10.1016/j.msec.2020.110963.

[2] D. Liu, L. Liang, J. M. Regenstein, and P. Zhou, "Extraction and characterisation of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (Hypophthalmichthys nobilis)," *Food Chemistry*, vol. 133, no. 4, pp. 1441–1448, Aug. 2012, doi: 10.1016/j.foodchem.2012.02.032.

[3] S.-K. Kim and E. Mendis, "Bioactive compounds from marine processing byproducts – A review," *Food Research International*, vol. 39, no. 4, pp. 383–393, May 2006, doi: 10.1016/j.foodres.2005.10.010.

[4] A. Mariod and H. Fadul, “Review: Gelatin, source, extraction and industrial applications,” *ACTA Scientiarum Polonorum Technol. Aliment.*, vol. 12, pp. 135–147, Jun. 2013.
[5] R. Ahmed, M. Haq, and B.-S. Chun, “Characterization of marine derived collagen extracted from the by-products of bigeye tuna (Thunnus obesus),” *International Journal of Biological Macromolecules*, vol. 135, pp. 668–676, Aug. 2019, doi: 10.1016/j.ijbiomac.2019.05.213.
[6] A. W. Sulaiman and N. M. Sarbon, “Characterization of acid soluble collagen (ASC) and pepsin soluble collagen (PSC) extracted from shortfin scad (Decapterus macrosoma) waste,” *Food Res.*, vol. 4, no. 6, pp. 2272–2280, Oct. 2020, doi: 10.26656/fr.2017.4(6).267.
[7] N. Narain and M. L. Nunes, “Marine Animal and Plant Products,” in *Handbook of Meat, Poultry and Seafood Quality*, John Wiley & Sons, Ltd, 2012, pp. 156–172. doi: 10.1002/9781118352434.ch11.
[8] T. Nagai, M. Izumi, and M. Ishii, “Fish scale collagen. Preparation and partial characterization,” *International Journal of Food Science & Technology*, vol. 39, pp. 239–244, Mar. 2004, doi: 10.1111/j.1365-2621.2004.00777.x.
[9] P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, T. Nagai, and M. Tanaka, “Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (Priacanthus tayenus),” *Food Chemistry*, vol. 89, no. 3, pp. 363–372, Feb. 2005, doi: 10.1016/j.foodchem.2004.02.042.
[10] L. Wang, B. Yang, and X. Du, “EXTRACTION OF ACID-SOLUBLE COLLAGEN FROM GRASS CARP (*CTENOPHARYNGODON IDELLA*) SKIN,” *Journal of Food Process Engineering*, vol. 32, no. 5, pp. 743–751, Oct. 2009, doi: 10.1111/j.1745-4530.2008.00242.x.
[11] J. Zhang, R. Duan, C. Ye, and K. Konno, “ISOLATION AND CHARACTERIZATION OF COLLAGENS FROM SCALE OF SILVER CARP (HYPOPHTHALMICHTHYS MOLITRIX): COLLAGENS FROM SCALE OF SILVER CARP,” *Journal of Food Biochemistry*, vol. 34, no. 6, pp. 1343–1354, Dec. 2010, doi: 10.1111/j.1745-4514.2010.00439.x.
[12] U. Hartina, “Properties of collagen from barramundi (Lates calcarifer) skin,” p. 8.
[13] F. Yu *et al.*, “Optimization of Extraction Conditions and Characterization of Pepsin-Solubilised Collagen from Skin of Giant Croaker (Nibea japonica),” *Marine Drugs*, vol. 16, no. 1, p. 29, Jan. 2018, doi: 10.3390/md16010029.
[14] S. Faralizadeh, E. Z. Rahimabadi, S. H. Bahrami, and S. Hasannia, “Extraction, characterization and biocompatibility evaluation of silver carp (Hypophthalmichthys molitrix) skin collagen,” *Sustainable Chemistry and Pharmacy*, vol. 22, p. 100454, Sep. 2021, doi: 10.1016/j.scp.2021.100454.
[15] B. F. Pamungkas, S. Supriyadi, A. Murdiati, and R. Indrati, “Extraction and characterization of acid-soluble collagen and pepsin-soluble collagen from the dry scales of the striped snakehead (Channa striatus),” *undefined*, 2018, Accessed: Sep. 13, 2022. [Online]. Available: https://www.semanticscholar.org/paper/Extraction-and-characterization-of-acid-soluble-and-Pamungkas-Supriyadi/a0410f6900e044a07ffe64147c7ca602c6618540

[16] M. Jridi *et al.*, “Chemical and biophysical properties of gelatins extracted from alkali-pretreated skin of cuttlefish (Sepia officinalis) using pepsin,” *Food Research International*, vol. 54, no. 2, pp. 1680–1687, Dec. 2013, doi: 10.1016/j.foodres.2013.09.026.
[17] T. Y. Ong, M. Shaik, and N. Sarbon, “Isolation and characterization of acid and pepsin soluble collagen extracted from sharpnose stingray (Dasyatis zugei) skin,” *Food Research*, vol. 5, Jun. 2021, doi: 10.26656/fr.2017.5(3).322.
[18] D. Wahyuni, “IKAN SELAR KUNING (Selaroides leptolepis),” p. 82.
[19] A. Febrianti, “HUBUNGAN PANJANG BERAT DAN FAKTOR KONDISI DI LAUT NATUNA YANG DIDARATKAN DI TEMPAT PENDARATAN IKAN PELANTAR KUD TANJUNGPINANG,” p. 8.
[20] “Produksi Perikanan.” https://statistik.kkp.go.id/home.php?m=prod_ikan_prov&i=2 (accessed Nov. 20, 2022).
[21] N. Alfiyani, N. Wulandari, and D. Adawiyah, “Validasi Metode Pendugaan Umur Simpan Produk Pangan Renyah dengan Metode Kadar Air Kritis,” *Jurnal Mutu Pangan : Indonesian Journal of Food Quality*, vol. 6, pp. 1–8, Apr. 2019, doi: 10.29244/jmpi.2019.6.1.1.
[22] L. Salvatore *et al.*, “Marine collagen and its derivatives: Versatile and sustainable bio-resources for healthcare,” *Mater Sci Eng C Mater Biol Appl*, vol. 113, p. 110963, Aug. 2020, doi: 10.1016/j.msec.2020.110963.
[23] A. M. E. Matinong, Y. Chisti, K. L. Pickering, and R. G. Haverkamp, “Collagen Extraction from Animal Skin,” *Biology (Basel)*, vol. 11, no. 6, p. 905, Jun. 2022, doi: 10.3390/biology11060905.
[24] A. Ramadhani, R. Riyanti, V. Wanniatie, and D. Septinova, “PENGARUH KOMBINASI SARIPATI BUAH NANAS DAN PEPAYA TERHADAP KUALITAS FISIK DAGING ITIK PETELUR AFKIR,” *JURNAL ILMIAH PETERNAKAN TERPADU*, vol. 8, p. 126, Dec. 2020, doi: 10.23960/jipt.v8i3.p126-132.
[25] L. Yang *et al.*, “Optimization of enzyme-assisted extraction and characterization of collagen from Chinese sturgeon (Acipenser sturio Linnaeus) skin,” *Phcog Mag*, vol. 9, no. 36, p. 32, 2013, doi: 10.4103/0973-1296.117859.
[26] H. Jafari *et al.*, “Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering,” *Polymers (Basel)*, vol. 12, no. 10, p. E2230, Sep. 2020, doi: 10.3390/polym12102230.
[27] S. N. H. Oslan, R. Shapawi, R. A. M. Mokhtar, W. N. Md. Noordin, and N. Huda, “Characterization of Acid- and Pepsin-Soluble Collagen Extracted from the Skin of Purple-Spotted Bigeye Snapper,” *Gels*, vol. 8, no. 10, p. 665, Oct. 2022, doi: 10.3390/gels8100665.
[28] Z. Song *et al.*, “Characterization and comparison of collagen extracted from the skin of the Nile tilapia by fermentation and chemical pretreatment,” *Food Chem*, vol. 340, p. 128139, Mar. 2021, doi: 10.1016/j.foodchem.2020.128139.
[29] X. Chen *et al.*, “Collagens made from giant salamander (Andrias davidianus) skin and their odorants,” *Food Chem*, vol. 361, p. 130061, Nov. 2021, doi: 10.1016/j.foodchem.2021.130061.
[30] S. Lin, Y.-P. Xue, E. San, T. C. Keong, L. Chen, and Y.-G. Zheng, “Extraction and Characterization of Pepsin Soluble Collagen from the Body Wall of Sea Cucumber

Acaudina leucoprocta," *Journal of Aquatic Food Product Technology*, vol. 26, no. 5, pp. 502–515, May 2017, doi: 10.1080/10498850.2016.1222560.
[31] R. Rosida, L. Handayani, and D. Apriliani, "Pemanfaatan limbah tulang ikan kambing-kambing (Abalistes stellaris) sebagai gelatin menggunakan variasi konsentrasi CH3COOH," *Acta Aquatica*, vol. 5, no. 2, pp. 93–99, Oct. 2018, doi: 10.29103/aa.v5i2.845.
[32] H. Cruz-López *et al.*, "Comparison of collagen characteristic from the skin and swim bladder of Gulf corvina (Cynoscion othonopterus)," *Tissue and Cell*, vol. 72, p. 101593, Oct. 2021, doi: 10.1016/j.tice.2021.101593.
[33] E. Pasaribu, T. Nurhayati, and M. Nurilmala, "Extraction and Characterization of Pepsin Enzyme from Tuna (Thunnus albacares) Gastric," *Jurnal PHPI*, vol. 21, no. 3, p. 486, Dec. 2018, doi: 10.17844/jphpi.v21i3.24727.
[34] P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, and F. Shahidi, "Isolation and characterization of collagen from the cartilages of brownbanded bamboo shark (Chiloscyllium punctatum) and blacktip shark (Carcharhinus limbatus)," *LWT - Food Science and Technology*, vol. 43, no. 5, pp. 792–800, Jun. 2010, doi: 10.1016/j.lwt.2010.01.006.
[35] S. Lin, Y.-P. Xue, E. San, T. C. Keong, L. Chen, and Y.-G. Zheng, "Extraction and Characterization of Pepsin Soluble Collagen from the Body Wall of Sea Cucumber Acaudina leucoprocta," *Journal of Aquatic Food Product Technology*, vol. 26, no. 5, pp. 502–515, May 2017, doi: 10.1080/10498850.2016.1222560.
[36] M. D. Shoulders and R. T. Raines, "Collagen Structure and Stability," *Annu. Rev. Biochem.*, vol. 78, no. 1, pp. 929–958, Jun. 2009, doi: 10.1146/annurev.biochem.77.032207.120833.
[37] A. Jongjareonrak, S. Benjakul, W. Visessanguan, T. Nagai, and M. Tanaka, "Isolation and characterisation of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (Lutjanus vitta)," *Food Chemistry*, vol. 93, no. 3, pp. 475–484, Dec. 2005, doi: 10.1016/j.foodchem.2004.10.026.
[38] S. Chen *et al.*, "Rapid isolation of high purity pepsin-soluble type I collagen from scales of red drum fish (Sciaenops ocellatus)," *Food Hydrocolloids*, vol. 52, pp. 468–477, Jan. 2016, doi: 10.1016/j.foodhyd.2015.07.027.
[39] J. Chen, L. Li, R. Yi, N. Xu, R. Gao, and B. Hong, "Extraction and characterization of acid-soluble collagen from scales and skin of tilapia (Oreochromis niloticus)," *LWT - Food Science and Technology*, vol. 66, pp. 453–459, Mar. 2016, doi: 10.1016/j.lwt.2015.10.070.
[40] Gita Syahputra, Hariyatun Hariyatun, Muhammad Firdaus, and Pugoh Santoso, "Extraction and Characterization of Collagen from Sand Sea Cucumber (Holothuria scabra)," *Jurnal Ilmu Pertanian Indonesia*, vol. 26, no. 3, p., 2021, doi: 10.18343/jipi.26.3.319.
[41] J. Wang, X. Pei, H. Liu, and D. Zhou, "Extraction and characterization of acid-soluble and pepsin-soluble collagen from skin of loach (Misgurnus anguillicaudatus)," *International Journal of Biological Macromolecules*, vol. 106, pp. 544–550, Jan. 2018, doi: 10.1016/j.ijbiomac.2017.08.046.
[42] G. Tegge, "Mitchell, J. R., and D. A. Ledward (Editors): Functional Properties of Food Macromolecules (Funktionelle Eigenschaften von Nahrungsmittel-Makromolekülen). Elsevier Applied Science Publishers, London and New York 1985. ISBN 0-85334-373-X. 433 Pages, with numerous figures and tables, hardcover)£ 52,—," *Starch - Stärke*, vol. 38, no. 8, pp. 287–287, 1986, doi: 10.1002/star.19860380811.

[43] W. Kemp, *Organic spectroscopy*. [Place of publication not identified] : Palgrave Macmillan, 2008. Accessed: Jun. 28, 2023. [Online]. Available: http://archive.org/details/organicspectrosc0000kemp_p0d8
[44] J. H. Muyonga, C. G. B. Cole, and K. G. Duodu, "Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (Lates niloticus)," *Food Chemistry*, vol. 86, no. 3, pp. 325–332, Jul. 2004, doi: 10.1016/j.foodchem.2003.09.038.
[45] "Wang et al. - 2018 - Extraction and characterization of acid-soluble an.pdf."
[46] D. Perkasa, D. Darwis, Erizal, and Tjahyono, "ISOLASI DAN SINTESIS GELATIN SISIK IKAN KAKAP PUTIH (LATES CALCARIFER) BERIKATAN SILANG DENGAN TEKNIK INDUKSI IRADIASI GAMMA," *Jurnal Sains Materi Indonesia*, vol. 14, pp. 40–46, Oct. 2012.
[47] S. Nalinanon, S. Benjakul, and H. Kishimura, "Collagens from the skin of arabesque greenling (Pleurogrammus azonus) solubilized with the aid of acetic acid and pepsin from albacore tuna (Thunnus alalunga) stomach," *Journal of the Science of Food and Agriculture*, vol. 90, no. 9, pp. 1492–1500, 2010, doi: 10.1002/jsfa.3973.
[48] A. Lancia, D. Musmarra, and M. Prisciandaro, "Kirk-Othmer Encyclopedia of Chemical Technology, Fourth Edition," 2002, pp. 582–602.
[49] C. A. Miles and A. J. Bailey, "Thermal denaturation of collagen revisited," *Proc. Indian Acad. Sci. (Chem. Sci.)*, vol. 111, no. 1, pp. 71–80, Feb. 1999, doi: 10.1007/BF02869897.
[50] D. Liu, P. Zhou, T. Li, and J. M. Regenstein, "Comparison of acid-soluble collagens from the skins and scales of four carp species," *Food Hydrocolloids*, vol. 41, pp. 290–297, Dec. 2014, doi: 10.1016/j.foodhyd.2014.04.030.
[51] S. Nalinanon, S. Benjakul, H. Kishimura, and K. Osako, "Type I collagen from the skin of ornate threadfin bream (Nemipterus hexodon): Characteristics and effect of pepsin hydrolysis," *Food Chemistry*, vol. 125, no. 2, pp. 500–507, Mar. 2011, doi: 10.1016/j.foodchem.2010.09.040.
[52] R. Yuqie, "Prosiding KIMIA FMIPA -ITS EKSTRAK GELATIN DARI TULANG RAWAN IKAN PARI (Himantura gerarrdi) PADA VARIASI LARUTAN ASAM UNTUK PERENDAMAN", Accessed: Mar. 30, 2022. [Online]. Available: https://www.academia.edu/5897535/Prosiding_KIMIA_FMIPA_ITS_EKSTRAK_GELATIN_DARI_TULANG_RAWAN_IKAN_PARI_Himantura_gerarrdi_PADA_VARIASI_LARUTAN_ASAM_UNTUK_PERENDAMAN
[53] I. Kołodziejska, E. Skierka, M. Sadowska, W. Kołodziejski, and C. Niecikowska, "Effect of extracting time and temperature on yield of gelatin from different fish offal," *Food Chemistry*, vol. 107, no. 2, pp. 700–706, Mar. 2008, doi: 10.1016/j.foodchem.2007.08.071.